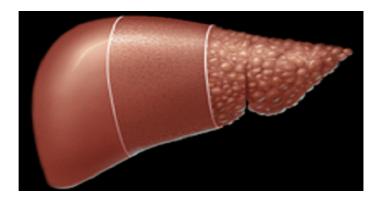
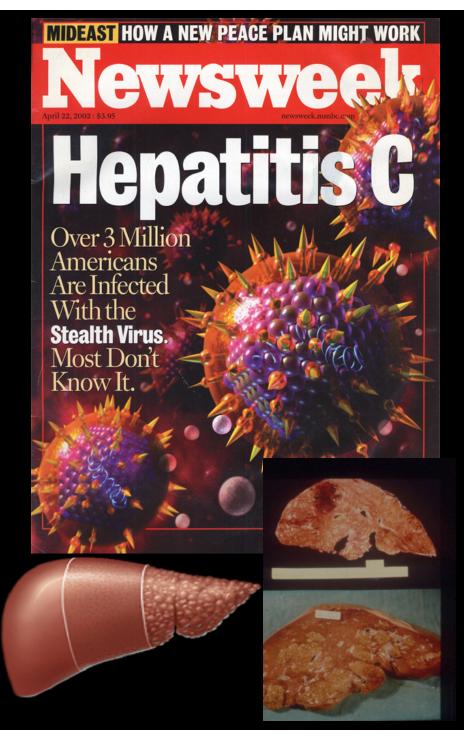
Hepatitis C Virus and Zero Day: A Hacker's View of the Virospace

T. Jake Liang, MD Liver Diseases Branch, NIDDK, NIH

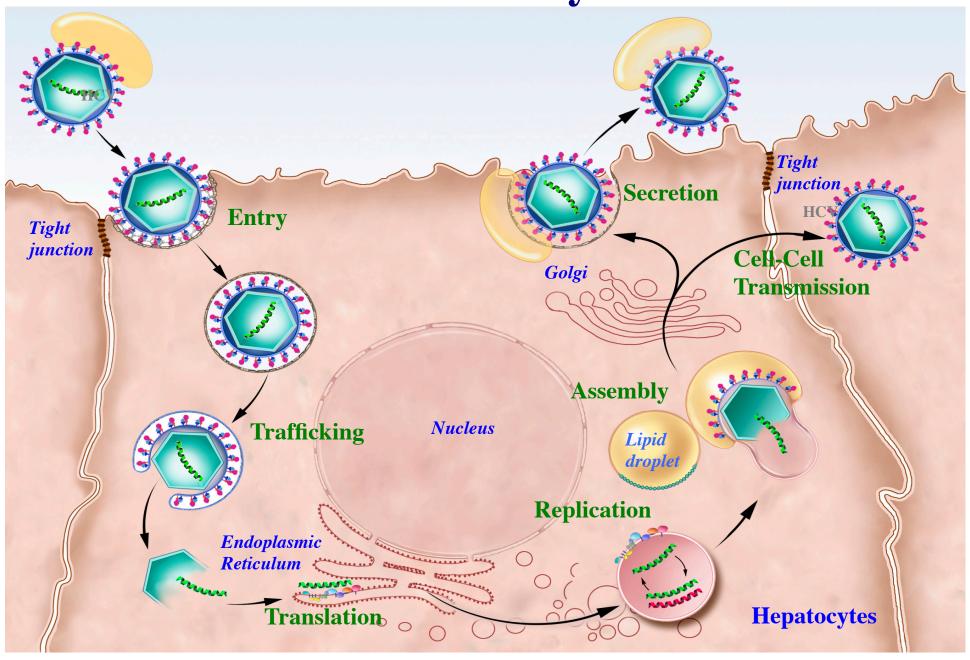






- Known as non-A non-B hepatitis for 20 years
- Discovered in 1989
- Flaviviridae family
- Positive single-stranded RNA genome
- Six genotypes with up to 30% sequence variations
- High mutational rates, quasipecies
- High rate of chronicity
- Silent disease progression
- No vaccine
- Treatment improving but not optimal

HCV Life Cycle

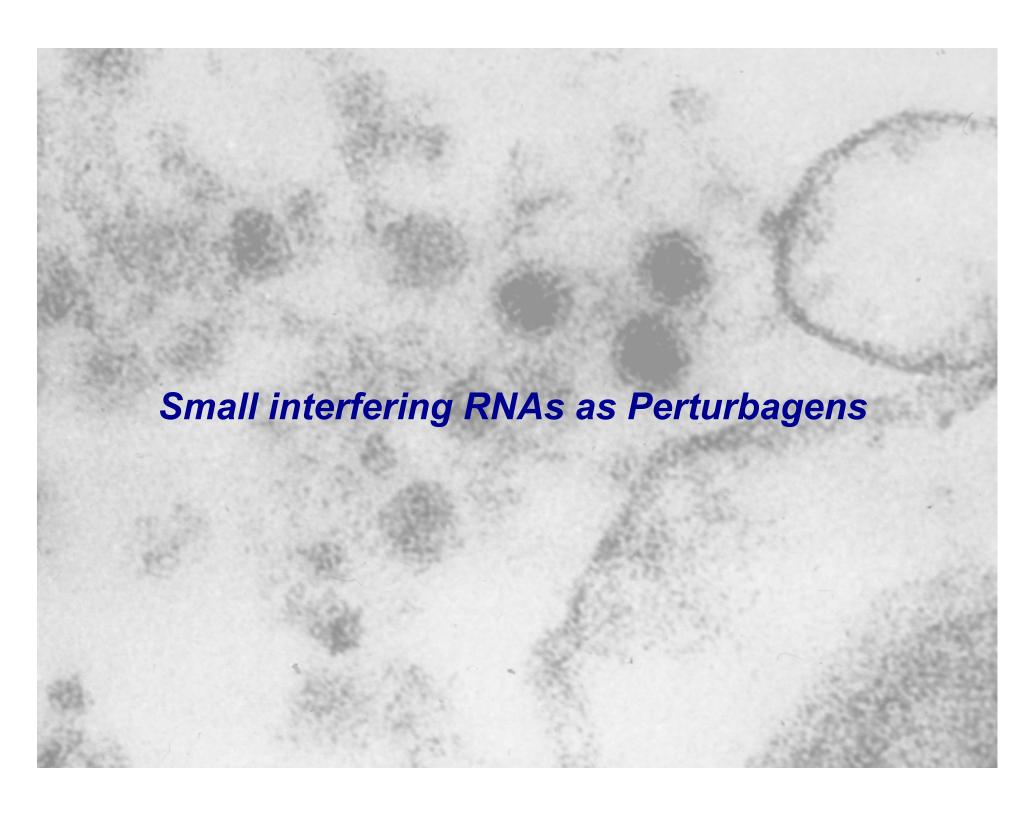


How about a zero day for HCV?

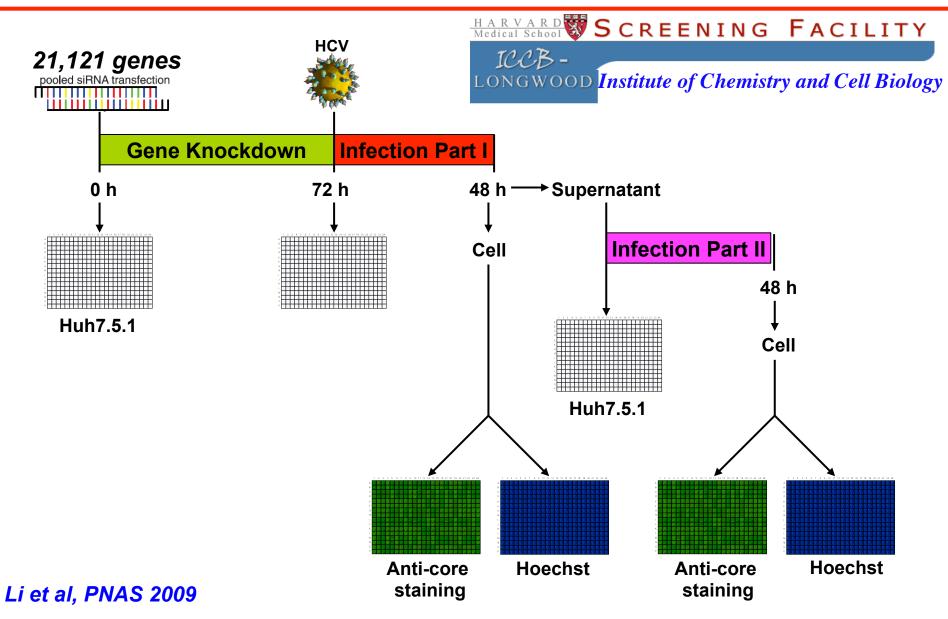
"In the face of great complexity, randomness is not an irrational process."

Gertrude Elion, Nobel Laureate

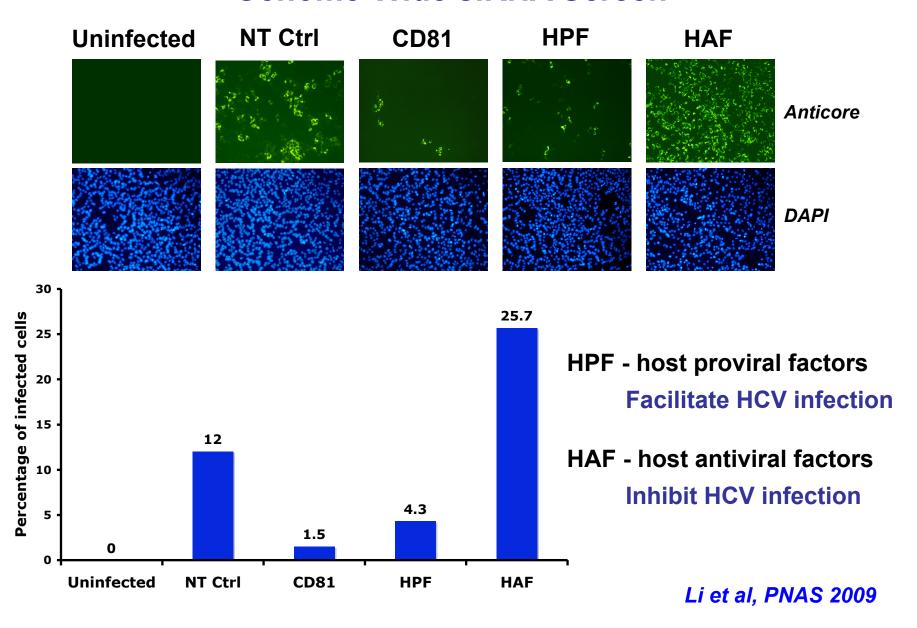
- Introduction of random "perturbagens" into virus-infected cells to force the virus to behave differently in order to reveal its secrets and identify its vulnerability
 - Small interfering RNAs
 - MicroRNAs
 - Chemical probes



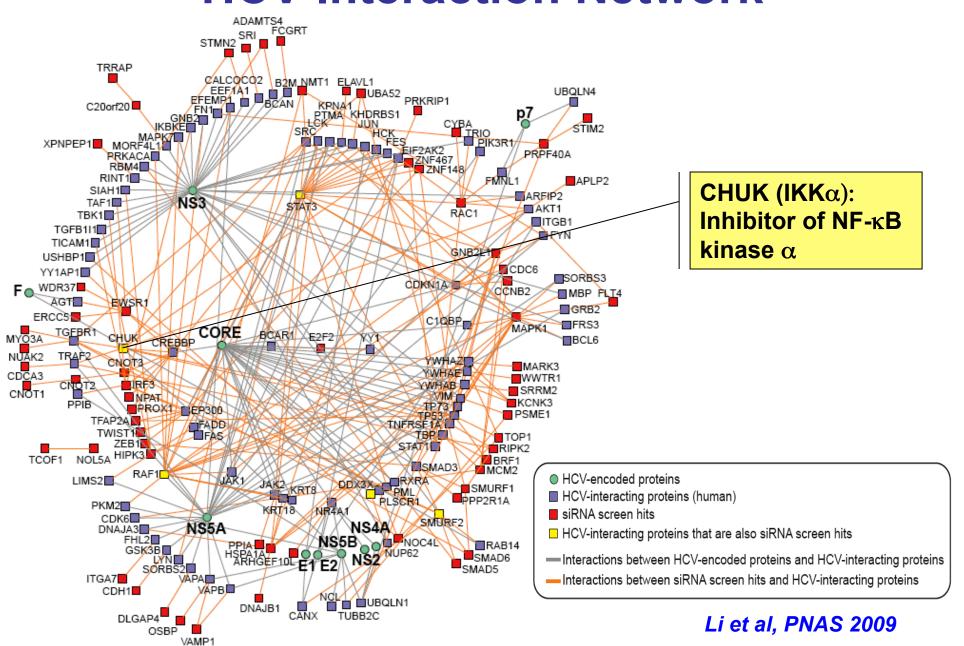
Global Analysis of HCV-Host Interactions: Genome-Wide siRNA Screen



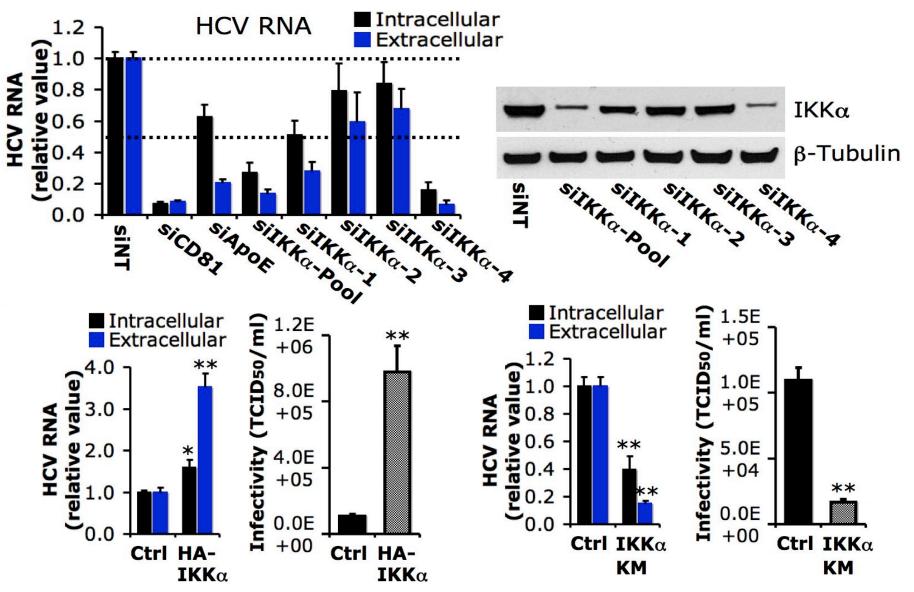
Global Analysis of HCV-Host Interactions: Genome-Wide siRNA Screen



HCV Interaction Network

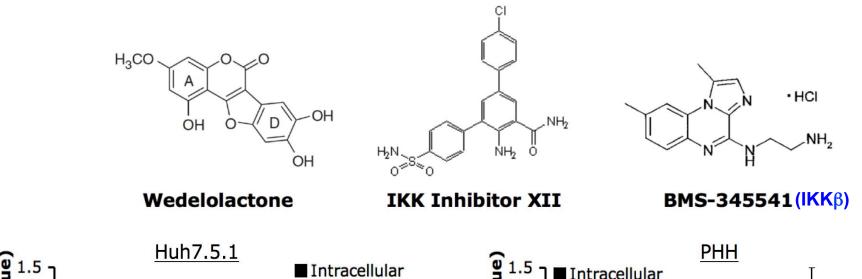


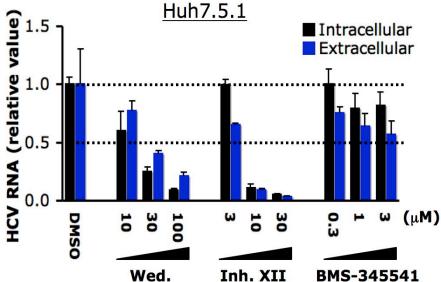
IKK α is Required in HCV Propagation

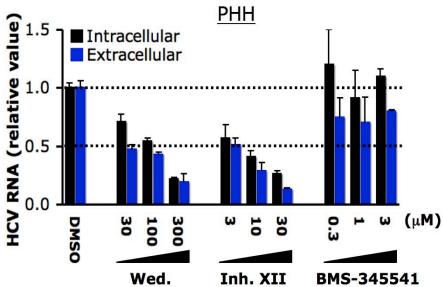


Li et al, Nat Med, June 2013

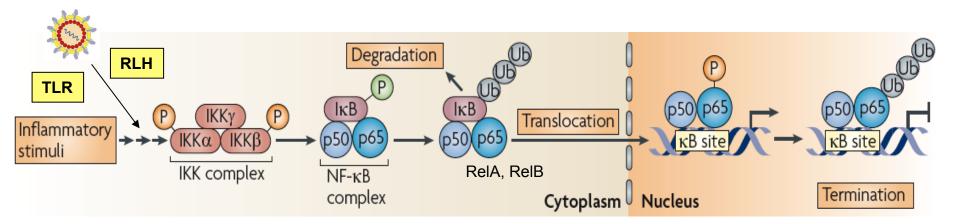
Effect of IKK Inhibitors on HCV Infection







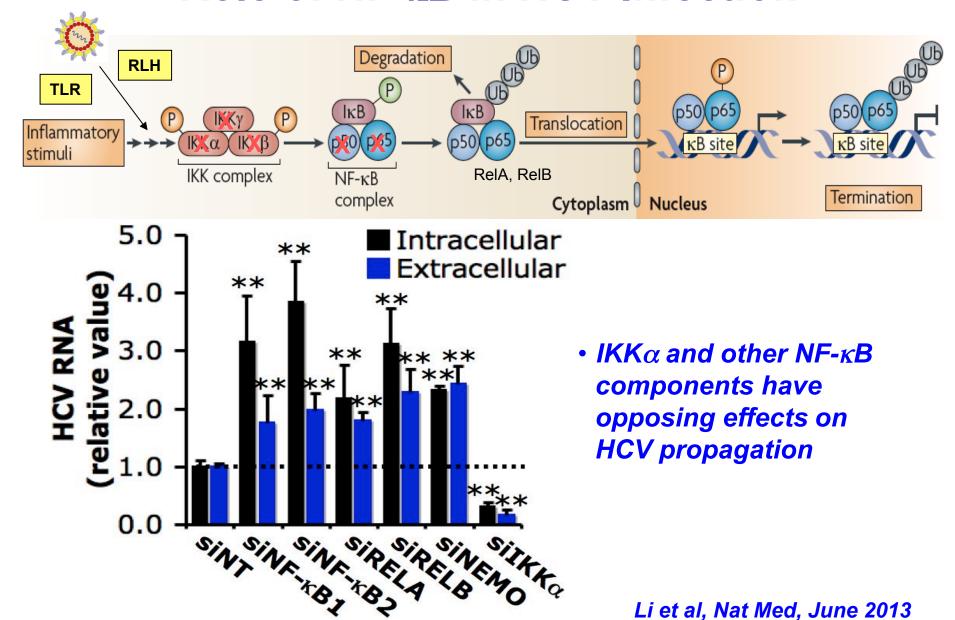
NF-kB Pathway



Gosh & Hayden Nat Rev Immunol 2008

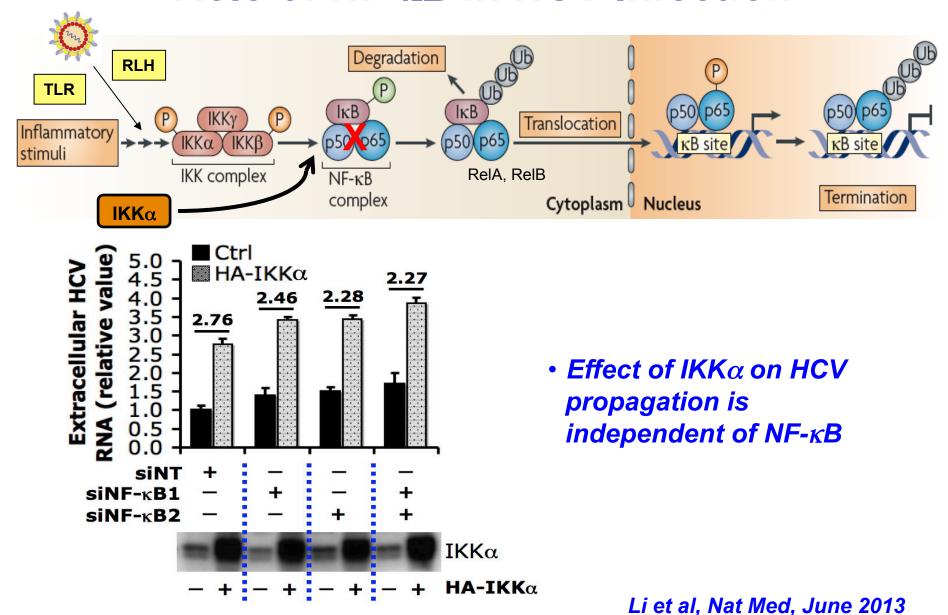
- Antiviral response (IRF3, IRF7, IRF9): induction of IFN pathway
- IKKβ & IKKγ (NEMO) in canonical NF-κB activation
- IKKα (CHUK) in non-canonical pathway (NF-κB2)

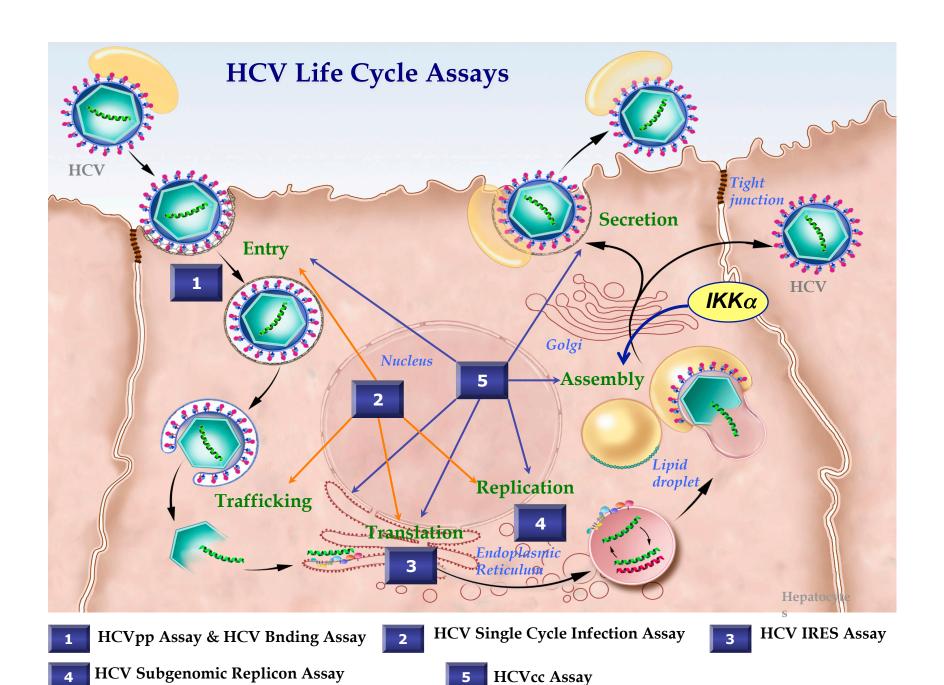
Role of NF-κB in HCV Infection



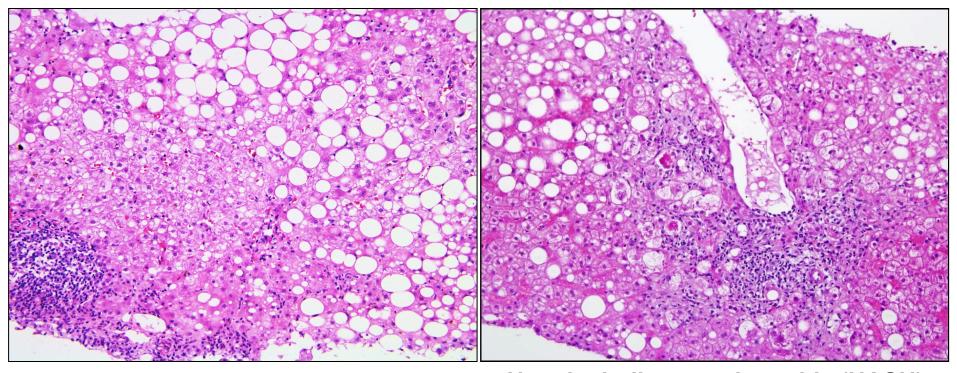
Li et al, Nat Med, June 2013

Role of NF-kB in HCV Infection



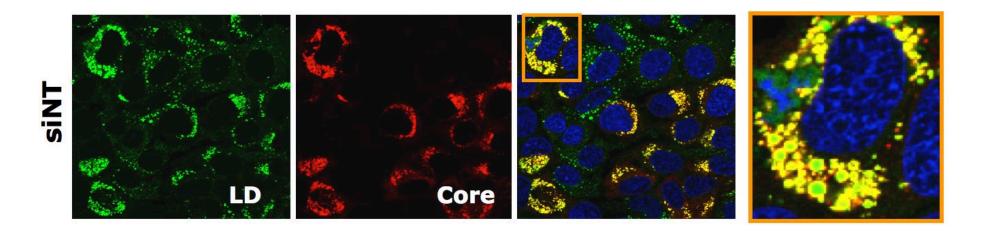


HCV and **Steatosis**



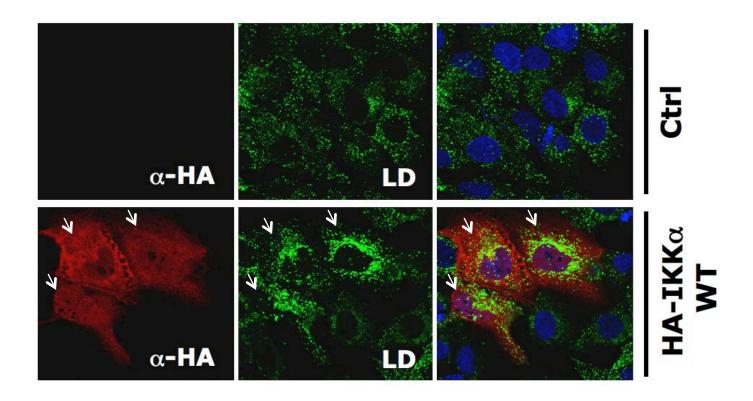
Nonalcoholic steatohepatitis (NASH)

Lipid Droplet Formation and IKK α

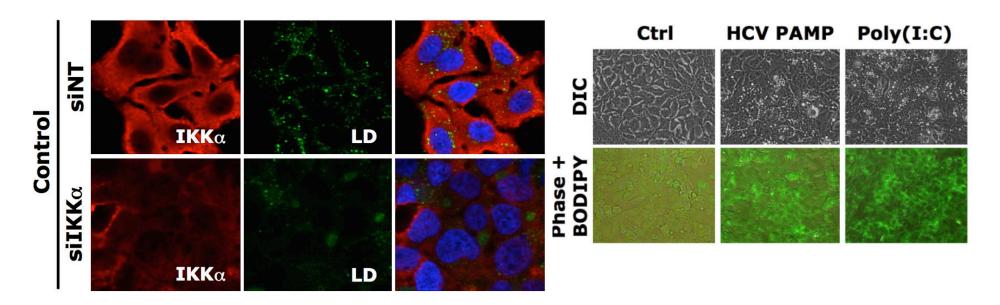


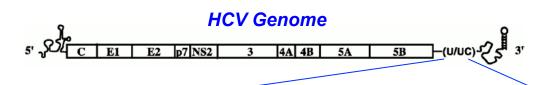
- HCV infection induces LD formation that co-localizes with HCV core
- IKK α mediates the effect of HCV on LD formation

Effect of IKK α on LD Formation



Mechanism of LD Biogenesis in HCV Infection



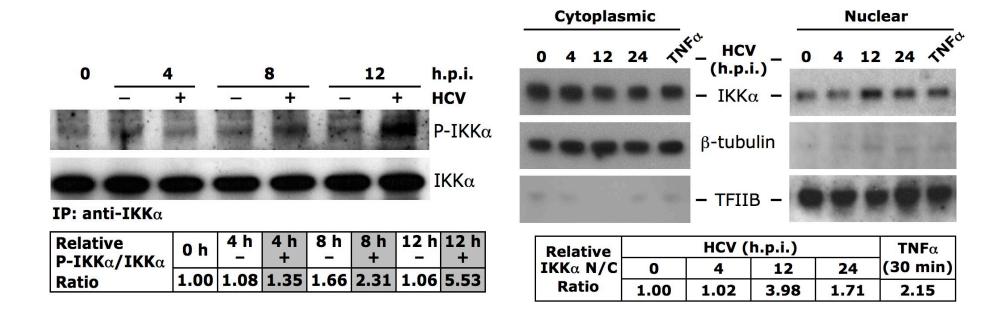


Pathogen-Associated Molecular Pattern

- Binds to RIG-I and activates IRF3
- Induces interferon-β production (Saito et al, Nature 2008)

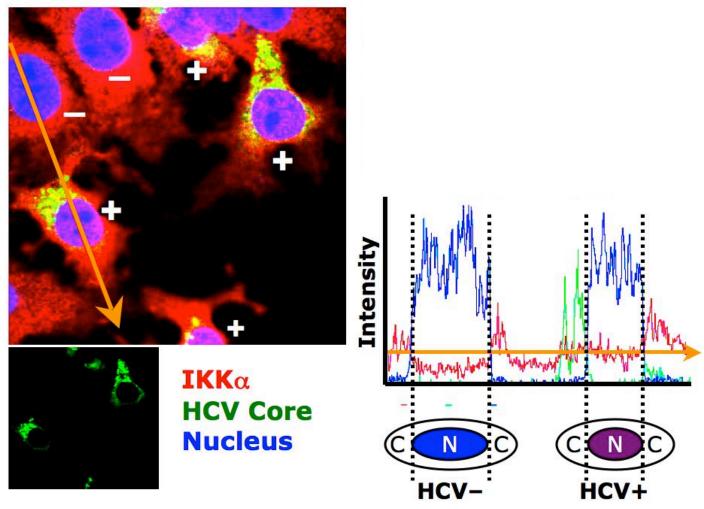
- IKKα is involved in LD biogenesis
- HCV induces LD biogenesis that is mediated by IKKlpha
- The HCV PAMP is responsible for this effect

Effect of HCV Infection on IKKα



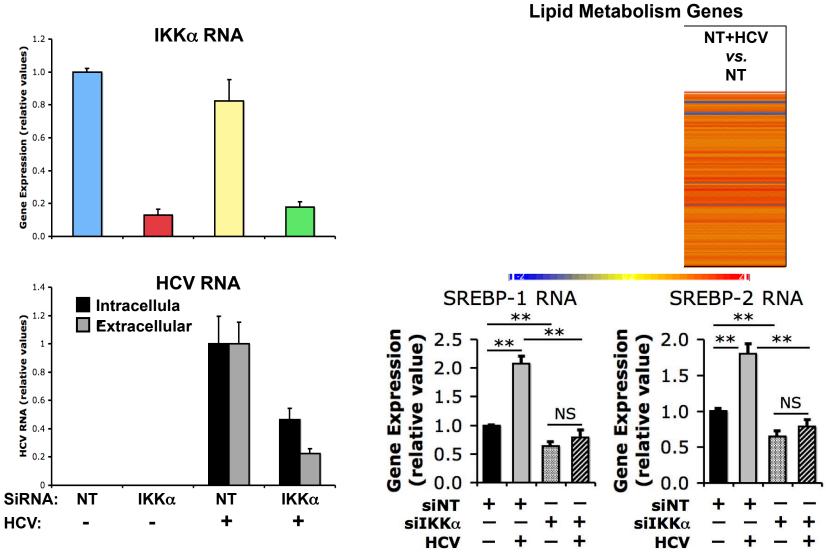
 HCV infection results in IKKα phosphorylation and nuclear localization

Localization of IKK α in HCV Infected Cells



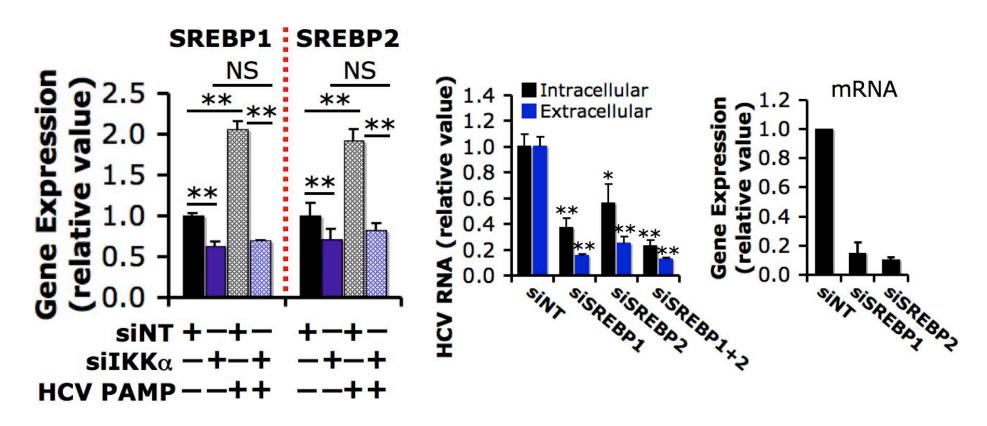
HCV induces nuclear translocation of IKKα

Role of IKKα in HCV Infection by Microarray Gene Expression Analysis



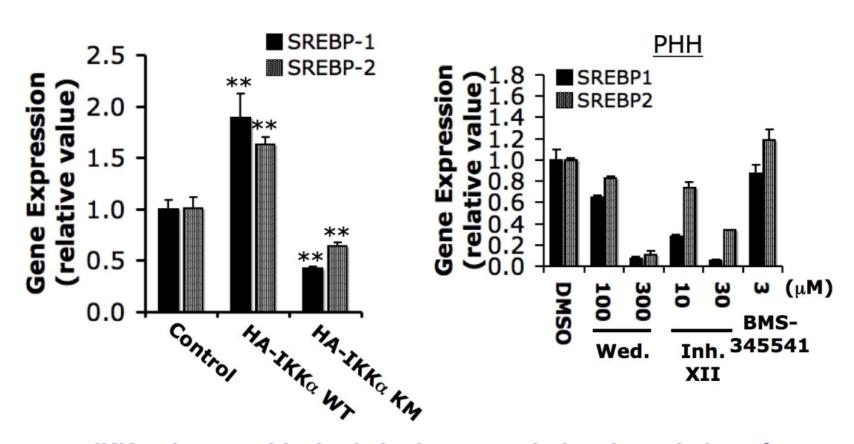
• HCV infection induces IKK α -mediated expression of lipid metabolism genes including SREBP-1 and -2

Role of SREBPs in HCV Infection



- HCV PAMP induces expression of SREBP-1 and -2 and other lipid metabolism genes
- SREBP-1 and -2 are important for HCV propagation

Role of IKK α in SREBP Expression



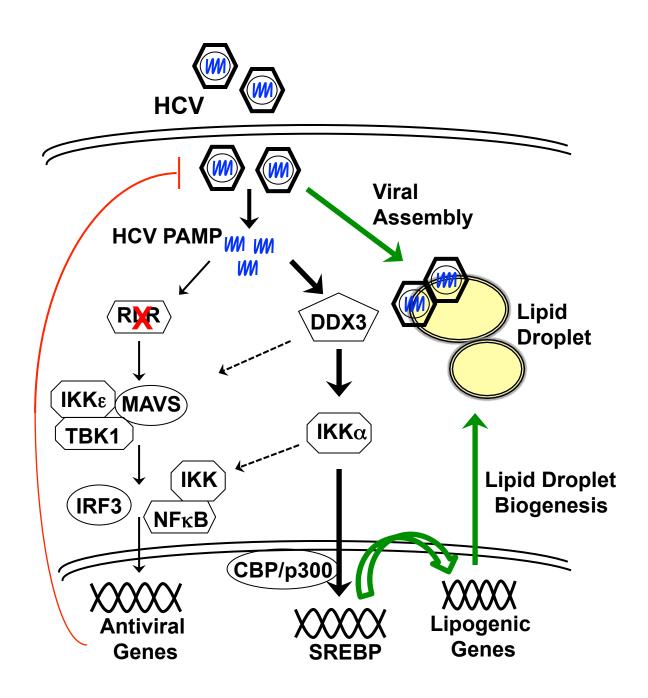
• IKK α plays a critical role in the transcriptional regulation of SREBP-1 and -2

Summary & Conclusion

- Genome-wide screen identifies novel host factors required for HCV infection and propagation
- One of the novel host pathways in HCV life cycle involves IKK α
- Unlike the other factors in NF- κ B pathway that have antiviral effects, IKK α exerts a predominantly proviral effect
- IKKα plays an important role in the assembly step of HCV life cycle

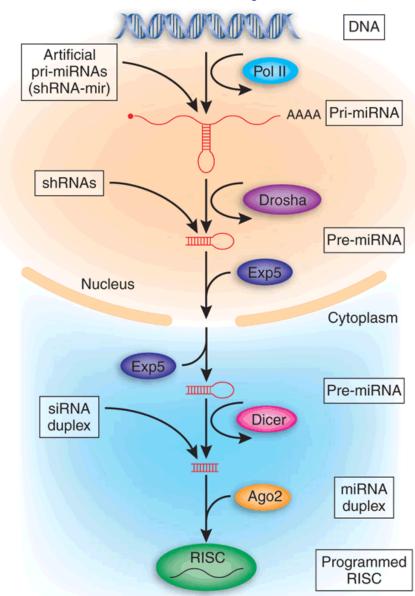
Summary & Conclusion

- HCV infection activates IKK α , which transcriptionally induces the expression of SREBP-1 and -2
- Activation of lipogenic genes and lipid droplet formation by SREBPs facilitates HCV assembly
- HCV exploits the host immune response and hijacks host lipid metabolism to its advantage
- Identification of novel host pathways in HCV life cycle has major implications in anti-HCV therapeutics



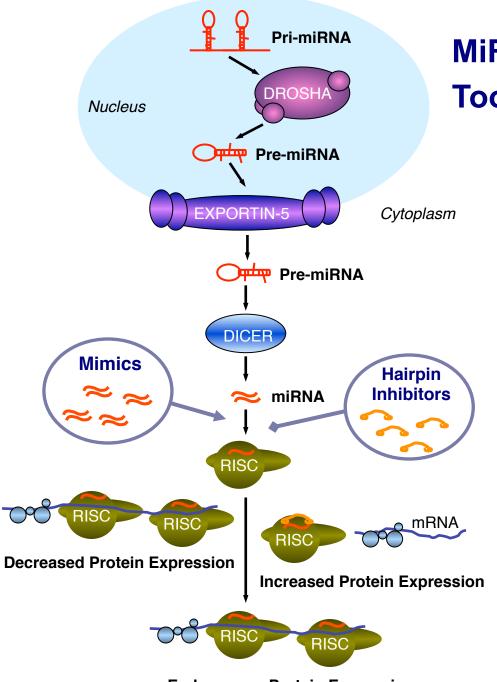
MicroRNAs as Perturbagens

MicroRNA Biogenesis Pathway

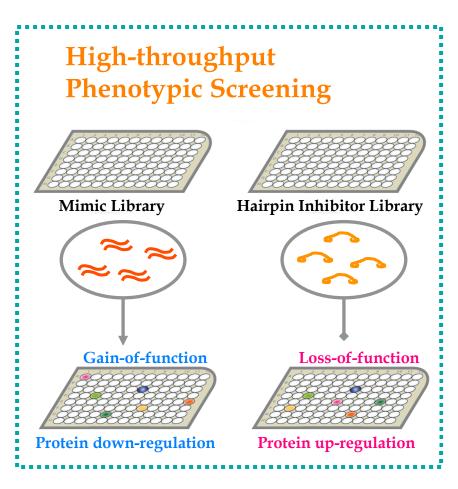


MicroRNA in Viral Infections

- Viral miRNAs affect host gene expression
- Host miRNAs modulate viral gene expression & life cycle
- MiR-122 plays a critical role in HCV replication (Jopling et al, Science 2005)
- Inhibitor of miR-122 is being developed as anti-HCV therapy (Lanford et al, Science 2010; Janssen et al, NEJM 2013)
- Other miRNAs implicated in HCV life cycle via regulation of host factors (Hou et al, Hepatology 2010; Banaudha et al, Hepatology 2011)

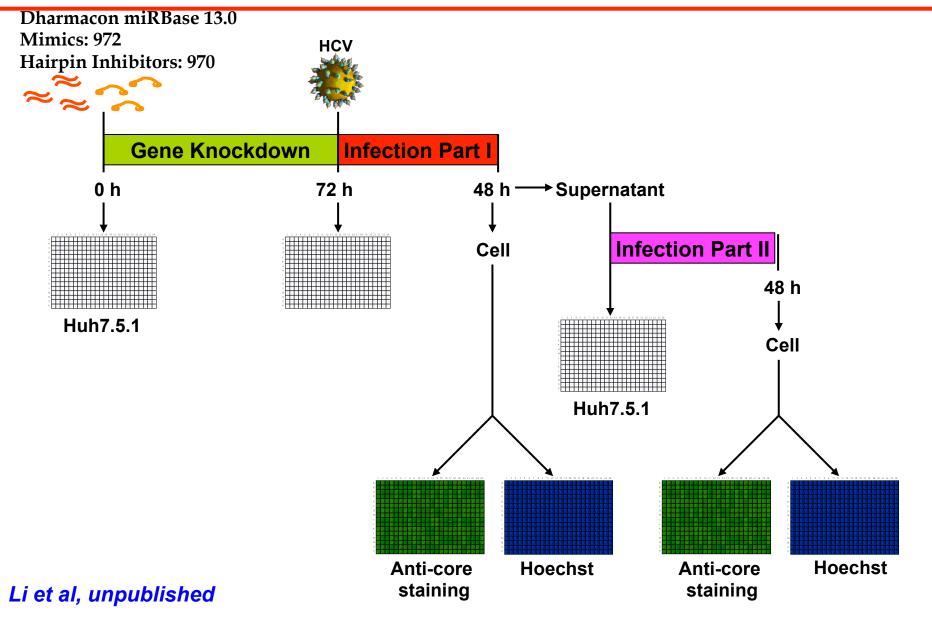


MiRNA Mimics and Inhibitors as Tools to Explore Biology

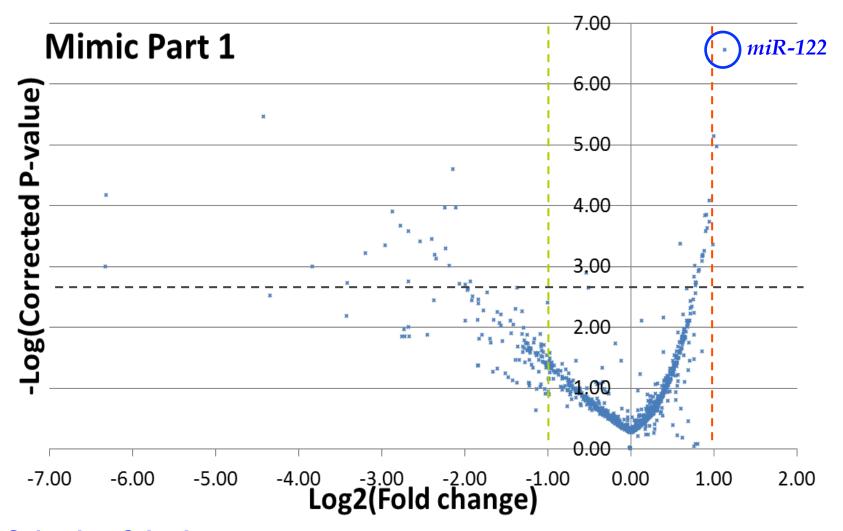


Endogenous Protein Expression

Global Analysis of HCV-Host Interactions: Genome-Wide miRNA Screen



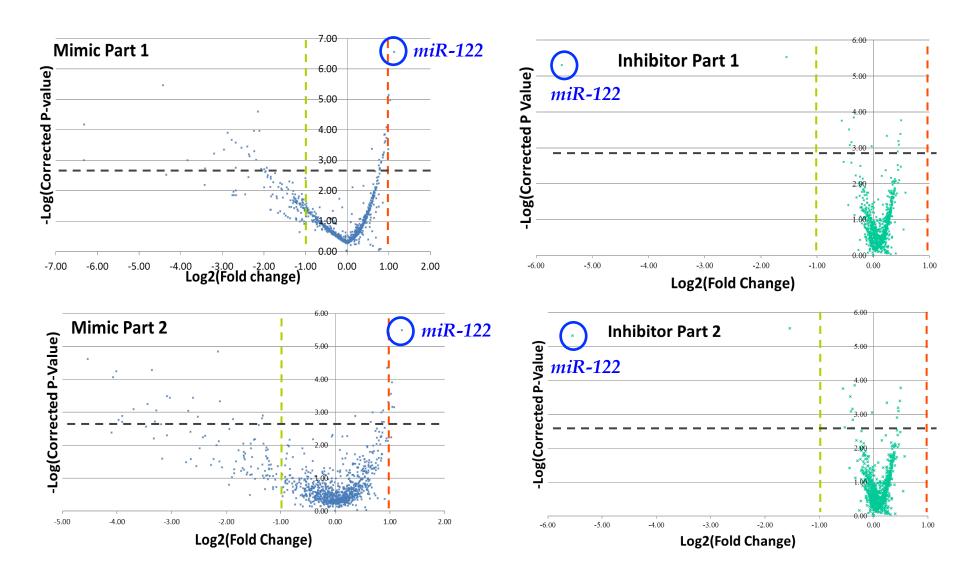
Global Analysis of HCV-Host Interactions: Genome-Wide miRNA Screen

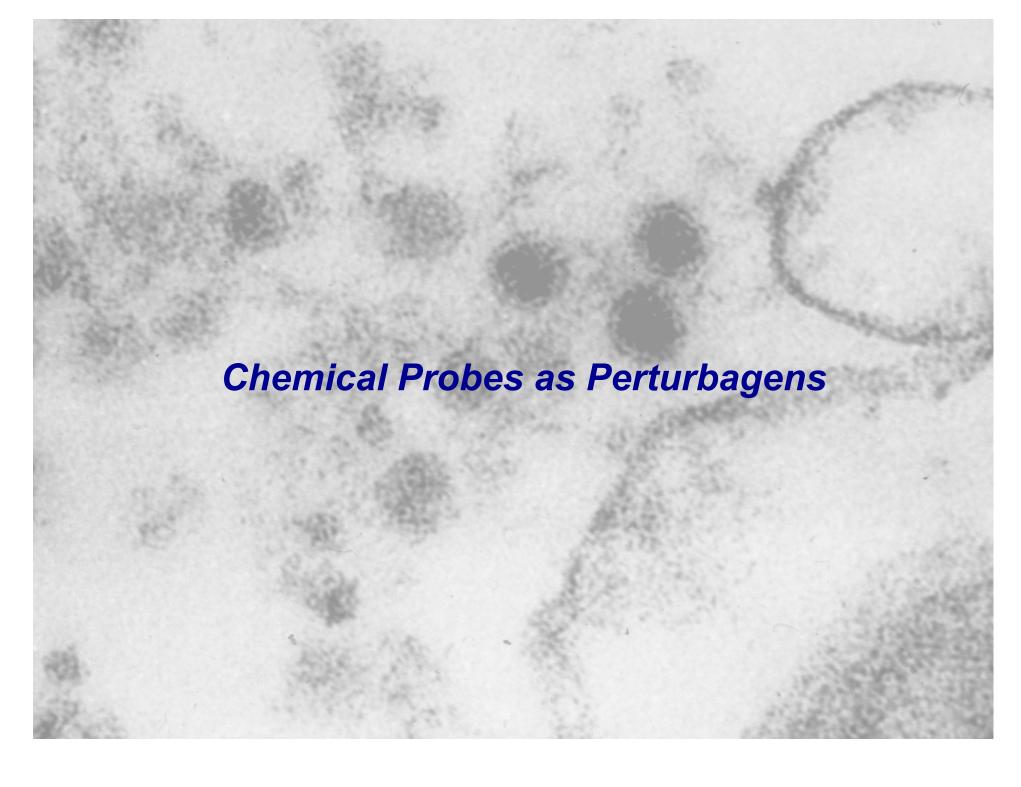


Selection Criteria

- Fold-increase/decrease ≥ 2 folds & P < 0.0025
- Viable cell numbers not decreased by ≥50% from the plate mean

Global Analysis of HCV-Host Interactions: Genome-Wide miRNA Screen

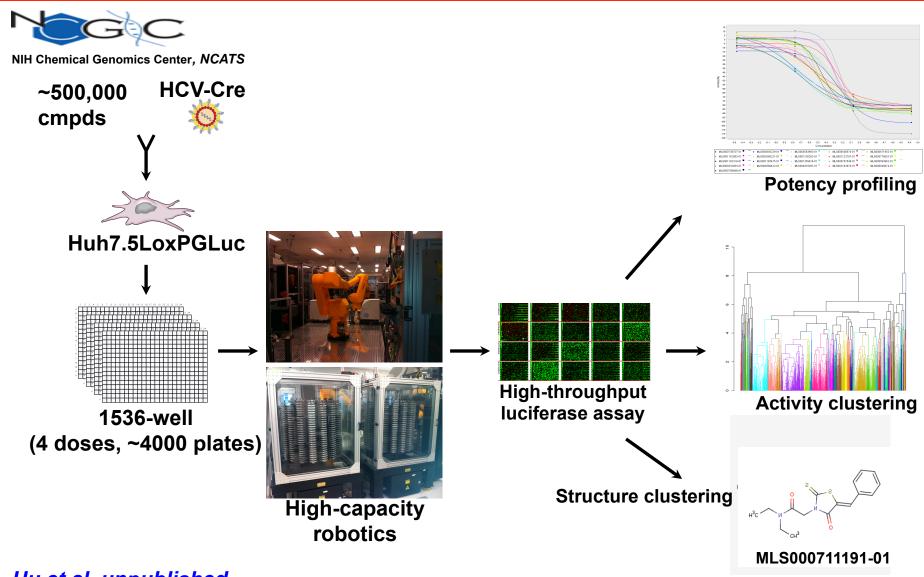




Optimization of Cell-Based Assays for High-Throughput Screen

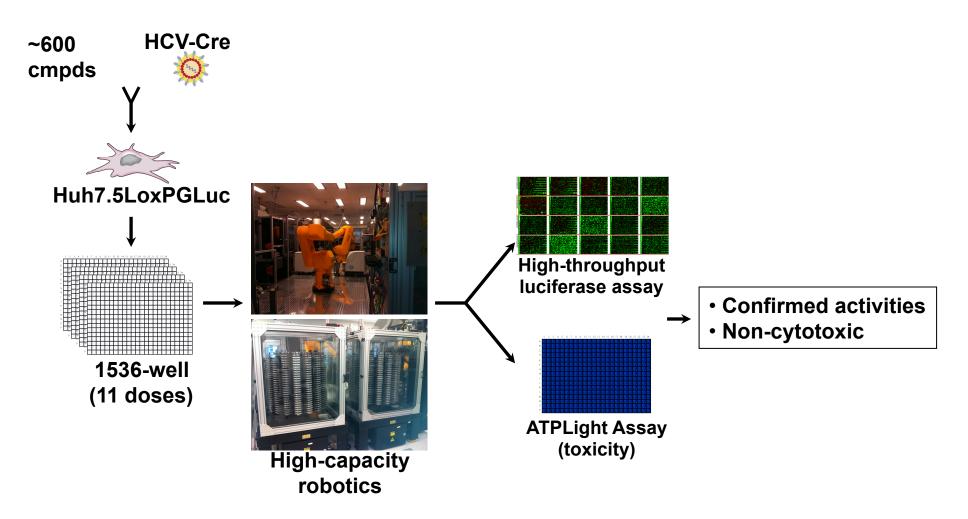
- Simplicity
- Minimal manipulation
- 1536-well plate format
- High signal-to-noise ratio
- Robust reproducibility (Z-factor>0.5 & coefficient of variation<30%)
- Quantitative HTS format: titration-based approach (Inglese et al, PNAS 2006)
- Cytotoxicity

Quantitative High-Throughput Screen of Small Molecule Chemical Libraries: Primary Screen



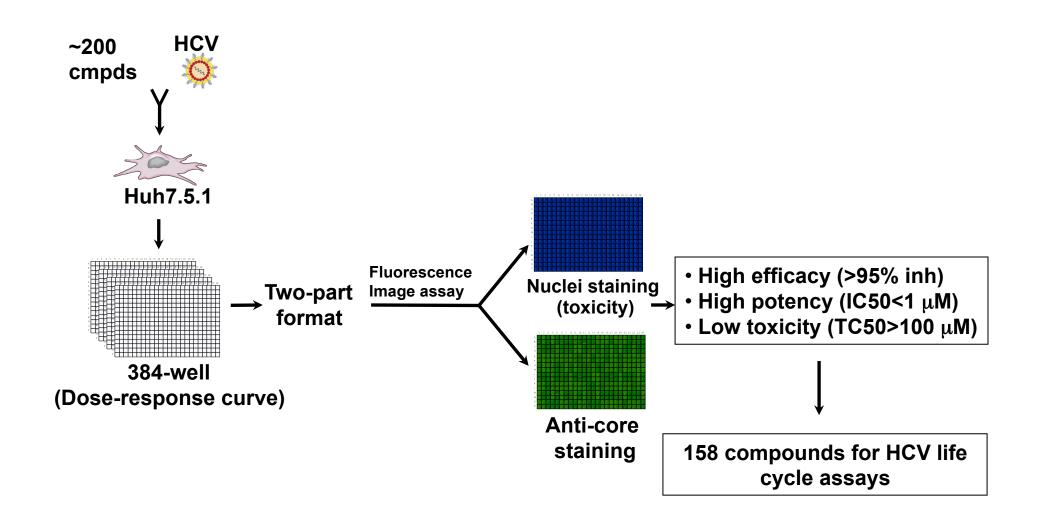
Hu et al. unpublished

Quantitative High-Throughput Small Molecule Library Screen: Confirmatory Screen



Hu et al. unpublished

Quantitative High-Throughput Small Molecule Library Screen: Secondary Screen



Quantitative High-Throughput Small Molecule Library Screen

- Selection of Lead Compounds
 - –High potency & efficacy
 - –No toxicity
 - -Targeting defined stage of HCV life cycle
 - -Structural bioinformatics
 - Drug-like properties
 - Novel structural classes

• 5 lead compounds (1 targeting entry, 1 replication, 3 assembly) selected for SAR, chemistry and further development

Are we at zero day for hepatitis C yet?

Not yet, but we are close!

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Hepatitis C: The End of the Beginning and Possibly the Beginning of the End

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